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Comparative Mesothelioma Induction in Rats by Asbestos and Non-Asbestos Mineral Fibers Possible Correlation with Human Exposure Data

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COMPARATIVE MESOTHELIOMA INDUCTION IN RATS BY ASBESTOS AND NON-ASBESTOS MINERAL FIBERS: POSSIBLE CORRELATION WITH HUMAN EXPOSURE DATA

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ABSTRACT

Fischer 344 rats were injected intrapleurally with chrysotile and crocidolite asbestos, erionite and attapulgite and observed for their entire lifespan. Detailed analysis of the number per unit mass of the various size categories of the fibers for each mineral species was determined and the dose in terms of actual number of mineral fibers computed. Data for survival time and number of tumors induced suggests that in addition to the length/width factors, an additional property plays a role in fiber potency. Analysis of the available data from the literature, where populations were known to have been exposed to pure mineral fibers, indicates that ranking for human mesothelioma incidence appears congruent with the rat intrapleural data.

INTRODUCTION

Since the intrapleural implantation experiments of Stanton, et al. (1), there has been a growing body of evidence that the length and width of mineral fibers play a determinate role in the induction of mesothelial tumors in the pleural or peritoneal cavities (1). Stanton's evidence appears quite clear on this and it it is generally assumed that his data show an optimal effect at length $\geq 8\mu m$ and width $\leq 0.25\mu m$. Since these data were mainly obtained from vitreous synthetic fibers of various sorts where fairly well defined length/width characteristics could be achieved, two questions have arisen; (1) do the same parameters hold for asbestos or asbestiform minerals which are chrystalline and infinitely variable in size and (2) is there a cut-off in length or width where these would no longer be an effect? Experiments with asbestos have appeared to show that when samples of short and long modal lengths of the same mineral species are employed experimentally, tumors predominate in the longer mode (2). These experiments are confounded, however, because while the mode of length may be different there is considerable overlapping in such specimens. Recent experiments using amosite where length can be controlled by ball milling showed clear cut reduction of tumorigenesis when the fibers did not exceed $5\mu m$ (3).

Despite the strength of the evidence that length-width ratio is the tumorigenic factor for mineral fibers, there has been a suspicion that persons exposed to crocidolite are more prone to develop mesothelioma than from exposure to chrysotile (4,5). A recent review of data from those instances where human exposure to pure fibers was certain has shown a ranking of crocidolite, amosite and chrysotile in decreasing order for mesothelioma induction (6). Furthermore, a striking preponderance of mesothelioma induction has been attributed to an

erionite fiber of the zeolite family (7). The propensity for this zeolite to induce mesothelioma as compared to asbestos has been confirmed by intrapleural, inhalation and intratracheal studies in rats (8,9,10).

The above facts suggest a hypothesis that some additional factor (other than length/width) may influence the degree of risk to mesothelioma. The determination of the presence of such a nongeometric factor and elucidation of its mechanism of action would not only be important in estimating human risk to environmental exposure to various asbestos and nonasbestos natural fibers but also would be of importance in evaluating hazard of new species of fibers proposed as asbestos substitutes. In the latter category, striking differences in the induction of pleural or peritoneal tumors have already been demonstrated in animal experiments (11). In order to test the above hypothesis, a series of experiments were undertaken employing natural mineral fibers of widely varying tumorigenic potential.

MATERIALS AND METHODS

Essential for this purpose was a very thorough minerological characterization in which the actual numbers of size and shape categories of mineral fibers were computed per unit mass of the minerals. The following fibers were chosen:

1) UICC chrysotile asbestos
2) UICC crocidolite asbestos
3) Erionite, a fibrous silicate of the zeolite class
4) Georgia Florida attapulgite, a fibrous clay mineral.

Two preparations of erionite from the same source (Rome, Oregon) supplied by Minerals Research, Clarkson, New York, were used in this study.

They were prepared by air elutriation (erionite W) and water sedimentation (erionite C), after being subjected to very brief milling.

Table I indicates the number per unit mass of mineral fibers of three size categories (a) all structures classed as fibers i.e. having lengths three times or more the width (aspect ratio = >3), (b) a short fine fiber less than 5 μm by 0.1μm or less (L=<5,W<.1), and (c) length 8μm or longer and width 0.25μm or less (L = >8, W<0.25). The latter class of fibers has been generally considered the most tumorigenic (1). It will be noted that there is a ranking from left to right in the number of fibers per unit mass. For example in the 8x0.25μm category there are 330 X more fibers for chrysotile than for erionite W and 126 X more than for crocidolite.

Included also are mean and median values for length, width and aspect rations (these values do not reflect numbers per unit mass).

Fischer 344 rats approximately three weeks of age were inoculated through the chest wall at the mid point of the right side of the thorax according to the schedule shown in Table 2 and held in a biological barrier for their entire life span.

RESULTS

For the purpose of this preliminary report descriptive statistical data only are presented. Data for dose effect and pathological considerations will be prepared in a later paper after pathological review and complete statistical analysis are completed.

Data for survival time determined by the Kaplan Meier method show ranking of survival from erionite W (shortest) to Attapulgite (longest). Summary of the data by mean and median survival time show that the effect of

the respective treatments differ widely (Table 3). Median values ranged from 369 days for erionite W to 789 days for crocidolite: Attapulgite was not different from pooled controls.

Tumor induction was generally conqruent with survival time ranging from 95% for mesothelioma for erionite to 1.4% for attapulgite, the latter being similar to controls (Table 4). The relationship of survival time to percent tumor is shown in Figure 1.

DISCUSSION AND CONCLUSIONS

As can be noted in Table I., a marked difference in the number of fibrous particles existed in our samples. If the prevailing view that long narrow fibers are rquired to induce mesothelial tumors (1), it would seem a reasonable assumption that the larger number of such fibers injected the greater the tumor induction and the less the survival time.

Our data does not give this expected result but to the contrary, the minerals with the fewest numbers of fibers (erionite) produced more tumor deaths and shorter survival time than either asbestos. Additionally, when either tumorigenesis or survival time is compared to the injected dose of mineral fibers, there is an inverse ratio between tumorigenesis and lifespan shortening and the number of mineral fibers. The data for both decrease in survival time and tumorigenesis when compared on the basis of the number of fibers ($L>8xW<0.25$) injected now rank in order of effect; erionite, crocidolite, and chrysotile.

When the relationship of the number of fibers to the effect either on survival time, expressed as reduction in life span, or percent tumor induction, a striking difference is noted in apparent potency of the mineral fibers per unit number. When such data is compared to data extracted from

the literature for a comparison of relative frequency of human mesothelioma from exposure to these same mineral fibers, there appears to be a striking similarity (Table 5). Table 6 shows the close comparative ranking of the experimental data from this experiment, and the data for human beings (where exposure is known to have been pure fibers) when compared either as percent mortality from mesothelioma or as a ratio of mesothelioma to lung cancer (corrected for SMR).

These data, therefore, would appear to argue for the presence of another chemical or physical agent (beyond length and width) as an additional factor in mesothelioma induction. The characterization of this factor may not only be of importance in evaluating the potential hazards of asbestos exposure but also may contribute significantly to understanding the striking differences in mesothelioma induction observed among certain synthetic fiber species.

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Table 1

ANALYSIS OF MINERAL FIBERS*

Parameter	Erionite (w)	Erionite (c)	Crocidolite (UICC)	Chrysotile (UICC)
Number of Fibers x 10^6/mg $A \geq 3$	850	1,080	3,360	21,000
Number of Fibers x 10^6/mg $L \leq 8$ µm, $W \leq 0.25$ µm	3.3	2.1	8.6	1,090
Number of Fibers x 10^6/mg $L \leq 5$ µm, $W \leq 0.1$µm	152	390	983	13,300
Mean Length (µm)	2.22	1.41	1.30	2.38
Median Length (µm)	1.60	0.99	0.82	1.08
Mean Width (µm)	0.25	0.17	0.16	0.10
Median Width (µm)	0.28	0.13	0.14	0.08
Mean Aspect Ratio (L/W)	10.76	10.27	7.95	29.55
Median Aspect Ratio (L/W)	8.00	7.31	5.94	11.53

* Analyzed by Dr. Philip Cook with transmission electron microscopy.
A = aspect ratio (L/W)
L = length
W = width

Table 2

Treatment Schedule

Test Substance	Dose in mg							Controls		
	0.5	2	4	8	16	32	Total	Untreated	SHAM	Total
Chrysotile	50	50	50	50	50	50	300	20	20	40
Crocidolite	50	50	50	50	50	50	300	20	20	40
Erionite W	50	50	50	50	50	50	300	20	20	40
Erionite C	50	50	50	50	50	50	300	20	20	40
Attapulgite	50	50	50	50	50	50	300	20	20	40

Table 3

Survival Time

	Fiber-Treated Animals			Untreated Controls			Saline Controls		
	# Studied	Mean	Median	# Studied	Mean	Median	# Studied	Mean	Median
Erionite W	299	372	369	20	818	843	20	887	878
Erionite C	299	482	462	20	820	848	20	879	913
Chrysotile	299	617	625	20	839	834	20	870	845
Crocidolite	299	769	789	20	812	801	20	801	829
Attapulgite	298	821	839	20	720	729	20	790	796
Controls				100	801	811	120	845	852

Table 4

Tumor Incidence in Fiber-Treated Rats

Test Substance	# Animals	# Tumors	% Tumors
Erionite W	144	137	95
Erionite C	144	123	85
Chrysotile	142	118	83
Crocidolite	144	65	45
Attapulgite	140	2	1.4
Controls (SHAM and Untreated)	79	1	1.3

*This report is based on pooled results of randomly selected (24) animals from each dose level of mineral sampled. Data represents animals dying with tumor.

Table 5

Proportion of Mesothelioma to Lung Cancer in Populations Exposed to Different Fibers

	Total Death Observed	Lung Cancer			Mesothelioma		
		Observed Number	Excess Number	% Excess	Observed Number	%	As Percent Excess Lung Cancer
Chrysotile	5500	407	99.0	1.8	12	.22	12.1
Amosite	861	144	85.5	9.9	19	2.2	22.2
Mixed Fiber	6751	960	485.3	7.2	320	4.7	65.9
Crocidolite	735	80	31.5	4.2	52	7.1	165.1
Erionite	67	8	5.0	7.4	27	40.0	540.0

Data for Asbestos from J.M. Hughes and Hans Weil Amer. Rev. Respiratory Disease, 1986, 133, 5–13.
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Data for Age Adjusted SMR for Lung Cancers in Turks kindly furnished by Dr. Artvinli..

Table 6

Relative Potency of Mineral Fibers in Mesothelioma Induction and Other Parameters Expressed as an Index (Erionite = 100)

	A	B	C	D	E
Erionite	100	100	100	100	100
Crocidolite	7.7	23.5	11.8	33	17
Chrysotile	0.2	0.3	2.5	2	5.5

A = Life shortening in rats – EPA Data (Dose by mineral fibers ≥ 8 ≤0.25μ)
B = Tumorigenesis from this study** EPA Data etc....
C = % difference from lung ca. – EPA Airborne Asbestos Health Assessment Update*
D = % difference from lung ca. – Hughs and Weil, 1986*
E = % mortality – Hughs and Weil, 1986*

*Data for erionite (C through F) taken from Artvinli and Baris, 1982
**Ratio number tumors/number fibers ≥ 8.0μm x ≤ 0.25μm

Figure 1 Comparison of Percent Rats with Tumor to Median Survival

(Both parameter pooled across dose range)